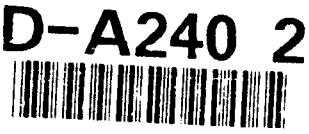

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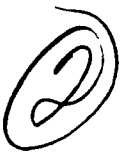

A TECHNIQUE FOR THE GROWTH OF EPIDERMAL SHEETS OBTAINED FROM PATIENTS UNDERGOING REDUCTION MAMMOPLASTY

FINAL REPORT

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RA 2; Growth Factors; Burns

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Research Progress Report

Project Number C122-89
MIPR 90MM0553

Title: A Technique for the Growth of Epidermal Sheets Obtained from Patients Undergoing Reduction Mammoplasty

Technical Approach: Discarded skin was obtained from patients undergoing reduction mammoplasty. The epidermis was enzymatically separated from the dermis. Keratinocytes were isolated from the epidermis and seeded in 25 cm2 cell culture flasks. The growth medium used was Keratinocyte Growth Medium (KGM) which has been developed for the growth of keratinocytes. In approximately 2 weeks the primary keratinocyte cultures were nearly confluent and were serially subcultured to expand the volume of cells.

When secondary cultures reached confluence, the cell medium was changed Dulbecco's Modified Eagles's medium containing 10% fetal calf serum. The change to a medium containing serum and a higher calcium concentration induced the keratinocytes to stratify into multi-layered sheets. These epidermal sheets were removed from the culture flask with Dispase, a neutral protease and attached to petrolatum gauze. At this point the sheets could be used as skin grafts.

Progress:

1. Establishment of culture technique at Clinical Investigation, BAMC. One of our goals for this project was to establish our keratinocyte culture technique at Clinical Investigation, BAMC. After procurement of the necessary supplies and an arrangement with Plastic Surgery for supplying human skin from breast reduction surgery, we were able to grow keratinocytes in culture. After numerous successful attempts of getting suitable epidermal sheets from human skin we were ready to provide autografts for patients.

2. Ultrastructural characterization of the epidermal sheet. The epidermal sheets were derived from skin from breast reduction surgery. The formation of the epidermal sheet was characterized by transmission electron microscopy. The number of cell layers

in the epidermal sheet was found to increase with time of incubation in serum supplemented media. The epidermal sheet reached a maximal thickness of 10-12 cell layers by 9-11 days in culture. Incubation of the sheets longer than 14 days resulted in the appearance of necrotic cells in the sheet. The keratinocytes in the sheets were characterized by the presence of numerous well formed desmosomes and intracellular tonofilaments. Melanocytes were also observed in the sheets. Hemidesmosomes were not observed between the basal layer of keratinocytes and the plastic substrate growth surface. Immunofluorescent staining revealed the presence of bullous pemphigoid antigen, keratin AE1:3 and type VII collagen.

This work was presented at the 1990 American Academy of Dermatology Annual Meeting. The title of the poster session was "Ultrastructural and Immunofluorescent Characterization of an In Vitro Human Epidermal Sheet Suitable for Grafting".

3. Grafting of patients. To date we have placed epidermal grafts on two patients. The first patient was a 5 year old boy with a diagnosis of junctional epidermolysis bullosa. This disease results in chronic skin erosions that are very resistant to conventional therapy. The grafting of this patient took place at Wilford Hall Medical Center. Epidermal allografts were derived from a skin biopsy from the patients mother. We have grafted this patient a total of 6 times. He has had significant healing of his central facial erosions. This work has been submitted and accepted for publication in the Journal of Dermatologic Surgery and Oncology.

The second patient was from the I.S.R. He was a civilian burn victim with 2nd and 3rd degree burns. We provided the I.S.R. with 5 epidermal autografts each with approximately 250 cm2 surface area. The grafts were left untouched for 2 weeks. After removal of the petrolatum gauze it was determined that the grafts did not take. We received a skin biopsy from a second burn patient but the cell cultures became contaminated and had to be discarded.

As of June 1990, we have not been able to provide autografts to the I.S.R. as I have started a Dermatology residency. I did not have any technical help over the past year. I did all the cell culture work for the project. We have applied for additional funds from the US Army R&D Command to hire a cell culture technician and work on the development of a composite in vitro skin graft. We believe that development of an artificial dermis as part of a composite in vitro graft is where our future efforts should be directed. We and our colleages in the I.S.R. feel that the epidermal grafts do not provide the ideal coverage for burn wounds. Although there are reports of successes with epidermal grafts in the medical literature, many investigators agree that an artificial dermis needs to be developed.